may be important for transducing signals downstream of Plexin A signaling, while the Rac sequestering mechanism may be important in transducing Plexin B signals. Hu et al. note that, unlike PlexB, PlexA does not interact directly with Rac1, which may suggest differences in signaling via these receptors. However, based on unpublished genetic evidence, the authors suggest that at least in flies PlexA and PlexB are likely to signal in a similar manner (see Figure).

Finally, it is worth noting that, although the study by Hu et al. focuses on direct interaction between PlexB and Rho family GTPases, evidence continues to accumulate that other molecules that regulate the activity of these GTPases also play important roles in specifying neuronal morphology. The most recent observations along these lines are decribed in an upcoming paper in *Cell* from the laboratory of Liqun Luo, demonstrating a role for p190 RhoGAP in axon branch stability in *Drosophila* (Billuart et al., 2001). Likewise, mice lacking p190 RhoGAP have axon guidance and fasciculation defects (Brouns et al., 2001), demonstrating the importance of RhoGAP in mammalian axon guidance.

The evidence linking Rho GTPases to axon guidance events is now quite compelling, but a number of challenges lie ahead. First, it remains to be determined how the various components that regulate Rho GTPase signaling act together to regulate coordinated cytoskeletal change in response to even a single guidance cue. Second, it is not clear if a common molecular mechanism is involved in mediating the response to different inhibitory guidance cues. These are difficult problems to address, but the progress to date is promising, and the papers by Winberg, Hu, and colleagues represent important advances toward these goals.

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Unwebbing the Presynaptic Web

The release of neurotransmitter from nerve terminals occurs at a specialized region of the presynaptic plasma membrane called the active zone. A dense matrix of proteins associated with the active zone, called the presynaptic web, is thought to play a fundamental role in defining these neurotransmitter release sites. In this issue of *Neuron*, Phillips et al. have identified conditions for the biochemical purification of the presynaptic web and show that the web is comprised of proteins involved in the docking, fusion, and recycling of synaptic vesicles.

Synaptic junctions, in particular those formed in the central nervous system, are characterized ultrastructurally by the presence of electron dense thickenings associated with the cytoplasmic faces of both the pre- and postsynaptic plasma membranes. The electron dense nature of these membrane specializations has been attributed to a high concentration of proteinaceous material that is readily visible by electron microscopy (EM) due to its very osmiophilic nature (see Peters et al., 1991). Early EM studies by Gray in the late 1950's suggested that synapses could be categorized either as type 1 or 2 depending upon the amount of electron dense material present at the postsynaptic plasma membrane (see Peters et al., 1991). For example, type 1 synapses exhibit a very pronounced electron dense thickening (~50 nm thick), referred as the postsynaptic density (PSD). These were subsequently shown to be primarily excitatory glutamatergic synapses. In contrast, type 2 synapses had a rather unremarkable PSD and were associated with inhibitory glycinergic or GABAergic synapses (see Peters et al., 1991). PSDs, viewed in cross-section, appear rather uniform in density and thickness from one edge of the junction to the other (see Peters et al., 1991). However, detailed ultrastructural analyses of purified PSDs have shown that PSDs exhibit a planar array of spherical units embedded in a filamentous lattice (see Matus and Taff-Jones, 1978). The PSD appears to be primarily involved in the clustering of both neurotransmitter receptors at high density and proteins involved in downstream signaling pathways. This concept is supported by extensive molecular, biochemical, cell biological, as well as physiological studies accumu-

lated over the last 10 years showing that macromolecular protein complexes are associated with specific transmitter receptor subtypes, promoting their postsynaptic localization and assembly into planar arrays (see Kennedy, 2000; Garner et al., 2000a; Sheng, 2001).

In contrast to the PSD, the electron dense material associated with the presynaptic plasma membrane in situ appears discontinuous with regular patches of electron dense material (~50 nm in diameter) flanked by clear regions. These electron dense patches are arranged in a regular network or grid situated just beneath the plasma membrane (Landis et al., 1988). A network of fine cytoskeletal filaments has been observed to connect these 50 nm patches with each other, creating a so-called presynaptic "web" or "grid." Fine filaments have also been seen to extend from this web deep into the presynaptic bouton as well as across the synaptic cleft into the PSD (Landis et al., 1988). Filaments traversing the cleft (Ichimura and Hashimoto, 1988) are thought to work in conjunction with synaptic adhesion molecules such as cadherins and N-CAM (Shapiro and Colman, 1998) to hold the presynaptic grid and the PSD in register, while those extending deep into the bouton are thought to retain/cluster the reserve pool of synaptic vesicles near the synaptic junction.

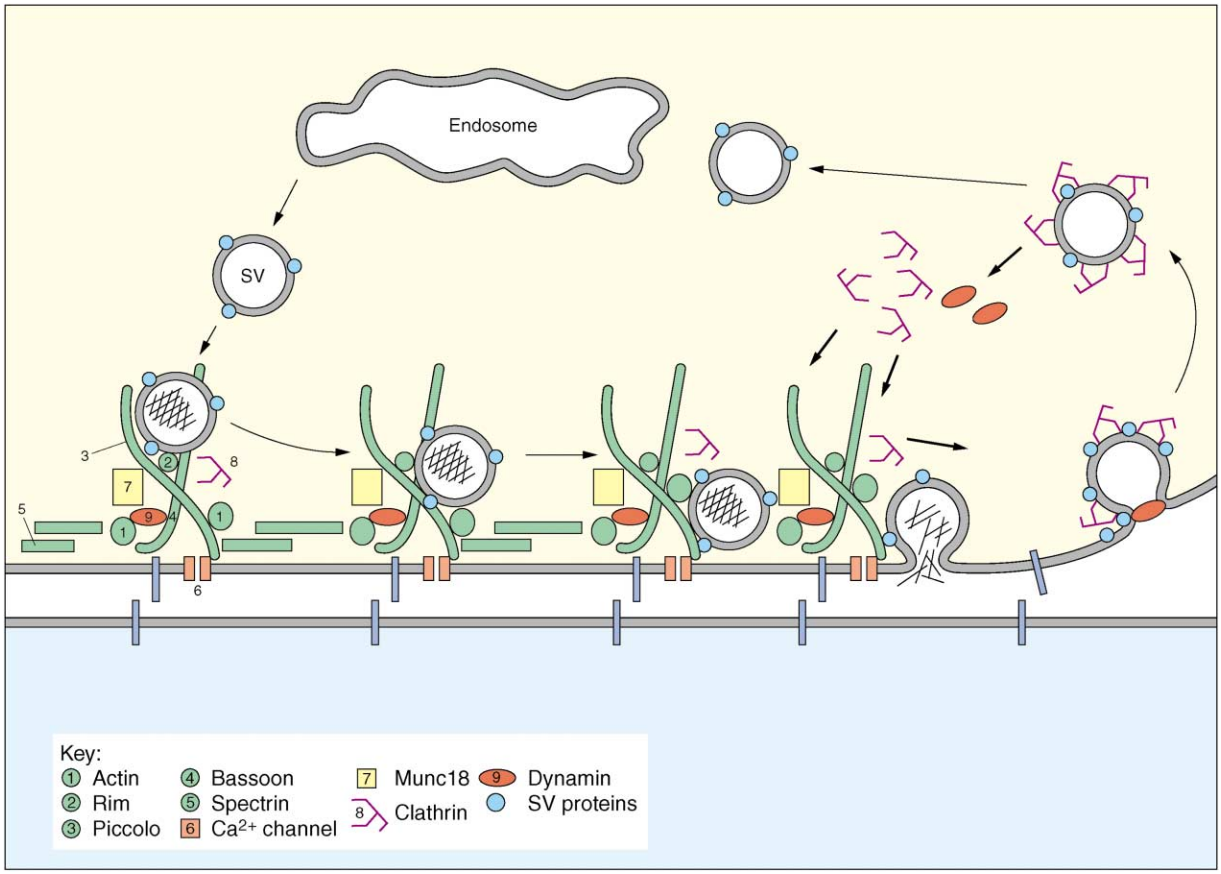
A basic function of presynaptic nerve terminals is the release of neurotransmitter. Here, action potentials reaching the nerve terminal stimulate the docking and fusion of neurotransmitter containing synaptic vesicles with a region of the presynaptic plasma membrane, called the active zone. The active zone is morphologically delineated by the presynaptic web and juxtaposed PSD (Dresbach et al., 2001). Subsequent to fusion, synaptic vesicle proteins are locally retrieved from the plasma membrane by endocytosis, repackaged into synaptic vesicles, and returned to the reserve pool of synaptic vesicles for future rounds of exocytosis (see Slepnev and De Camilli, 2000). The presynaptic web is thought to play a role in both of these events; however, its function at the molecular level is poorly understood. A role in synaptic vesicle exocytosis is suggested by EM studies showing that synaptic vesicles, docked at the active zone plasma membrane, occupy the space between the electron dense patches of the presynaptic web. Similarly, a role in synaptic vesicle endocytosis is suggested by data showing that endocytosis occurs at regions of the presynaptic plasma membrane directly flanking the presynaptic web. These observations raise the possibility that the web may also participate indirectly in synaptic vesicle endocytosis, perhaps by transiently sequestering the endocytic machinery near the active zone (see Slepnev and De Camilli, 2000).

Although molecular and genetic approaches in recent years have begun to identify the components of the presynaptic web (see Garner et al., 2000b; Dresbach et al., 2001), researchers have been hampered by the lack of biochemical methods for the purification of this structure. In this issue of *Neuron*, Phillips et al. (2001) present findings demonstrating that specific components of the presynaptic web can be biochemically purified and reassembled in vitro in a pH-dependent manner. These studies provide fundamental clues both to the composition of the web and to the intrinsic properties of its assembly.

In the early seventies, a number of groups employed sucrose density centrifugation and detergent extraction of synaptosome preparations to biochemically purify PSDs (Cotman et al., 1974; Matus and Taff-Jones, 1978). These preparations ultimately lead to the subsequent isolation and characterization of many of the currently known PSD proteins (Langnaese et al., 1996; Kennedy, 2000). Already at this time, it was appreciated that the purity of the PSD, as assessed by EM, was affected by the pH of the buffer. Utilizing this information, Phillips and colleagues have explored the conditions under which the presynaptic web remains associated with these PSD preparations. Ultrastructural analysis of these synaptic junctional preparations at a range of pH (6.0–8.0) revealed that the electron dense patches (or particles) of presynaptic web material were associated with the PSD at low pH (6.0) and were progressively lost at higher pH (7.0–8.0). At an intermediate pH, fibrils extending from the PSD that tethered together these particles could be seen. Morphogically, these purified junctions closely resemble synaptic junctions in vivo. Interestingly, the presynaptic web material, completely solubilized in pH 8.0 buffer and then slowly returned to pH 6.0 by dialysis, was found to aggregate into what appeared to be 50 nm electron dense particles tethered together by fine filaments. Excitingly, if purified PSDs were added to these solubilized presynaptic web preparations at pH 8.0, 50 nm particles were observed to become asymmetrically associated with the PSD when the pH of the mixture was slowly lowered to pH 6.0. That is to say, these web-derived particles appeared on only one face of the PSD. Morphologically, these reassembled synaptic junctions resembled synaptic junctional preparations isolated at pH 6.0. These are very fundamental findings and suggest that the presynaptic web can be dynamically disassembled and then reassembled in vitro. Moreover, this approach should allow factors that promote assembly of the presynaptic web to be explored.

Using their ability to purify presynaptic web material, Phillips and colleagues went on to identify the proteins present in these solubilized preparations using primarily mass spectrometry. The most prominent proteins included components of the endocytic machinery, e.g., clathrin and dynamin, constituents of the actin/spectrin cytoskeleton, as well as Munc18 and NSF, proteins involved in synaptic vesicle exocytosis. Also present, albeit at much lower levels as compared to the amount present in the insoluble PSD preparation, were N-cadherin, β-catenin, and γ-protocadherin, as well as the presynaptic active zone proteins RIM and Bassoon.

What does this body of data tell us about the presynaptic web, its properties, and potential functions? First, the data indicate that the cytoskeleton, in particular actin and spectrin, is a major component of the web. Importantly, actin and spectrin have been found in numerous examples to underlie the assembly of the membrane-associated cortical cytoskeleton into two-dimensional arrays. As such, these two proteins may represent the fibrillar nature of these isolated web preparations and perhaps also be important for the dynamic assembly of the web at active zones. RIM and Bassoon are also considered to be structural/cytoskeletal proteins (see Garner et al., 2000b). Both are large multidomain proteins, which in contrast to actin and spectrin, are found exclusively at presynaptic active zones. As such, they



Presynaptic Web and Associated Proteins

The presynaptic web is formed by a grid of cytoskeletal filaments and zones of electron dense patches/particles. Spectrins and actin could be part of the structural components of the filamentous presynaptic grid. The patches/particles of electron dense material could be a supramolecular structure formed by the presynaptic scaffold proteins piccolo/bassoon and associated exo-endocytotic proteins (i.e. Munc18, dynamin, clathrin). At these sites, exo-endocytotic proteins are retained to become readily available to synaptic vesicles. After the exo-endocytosis process has occurred, these proteins could be reassociated with this supramolecular complex to be reutilized in the synaptic vesicle cycle.

are excellent candidates to help organize the proteins associated with the active zone and the presynaptic web itself (Figure). Their role in the in vitro assembly of the purified presynaptic web material is presently unclear since at pH 8.0, both RIM and Bassoon remain primarily associated with the insoluble synaptic junctional/PSD preparations. Thus, it seems likely that these tightly associated presynaptic active zone proteins may provide the backbone or nucleation sites for the reassociation/reassembly of the electron dense presynaptic web material with the PSDs, as described in this paper (Phillips et al., 2001). From a functional perspective, the most interesting group of proteins present in these purified web preparations is NSF, Munc18, clathrin, and dynamin. All are considered to be soluble proteins, yet they perform fundamental roles in directing/regulating synaptic vesicle exo- and endocytosis. As such, they represent a class of proteins known to be critical for presynaptic function, but for which there are few clues as to how they are maintained at or near the active zone. Their association with the presynaptic web, perhaps in a dynamic manner, could provide a beautiful yet simple mechanism for keeping them at their site of action.

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Attention, Adaptation, and the Motion Aftereffect

Activation of the human visual motion area V5/MT was previously thought to be the basis of the motion aftereffect. New findings suggest that previous observations were confounded by attention and arousal, providing evidence that adaptation of directionally selective neurons in area V5/MT represents the fundamental substrate for the motion aftereffect.

Many striking visual illusions result from disturbances of the equilibrium of the visual system caused by brief periods of intense activation. For example, prolonged viewing of a stimulus moving in one direction causes a motion aftereffect (MAE); a stationary stimulus viewed subsequently appears to move in the opposite direction (Wolgemuth, 1911). This is often known as the "waterfall illusion," referring to the MAE experienced when looking at adjacent static rocks after gazing at a waterfall for a prolonged period. The physiological substrate and neural locus of MAEs is under active investigation. Traditionally, MAEs have been attributed to fatigue or adaptation of units in visual cortex selective for particular directions of motion. Attention has therefore focused on cortical area V5/MT, an area in visual cortex that responds well to visual motion, and whose homolog in monkey contains a high proportion of directionally selective neurons.

Recent functional imaging studies in humans have examined cortical activity when subjects are presented with a static stimulus following adaptation to unidirectional motion, and either asked to passively view or actively judge the duration of the ensuing motion aftereffect (Culham et al., 1999; Hautzel et al., 2001; He et al., 1998; Taylor et al., 2000; Tootell et al., 1996). Typically, under these conditions, V5/MT activity is found to be elevated relative to a control condition when no motion aftereffect is perceived, such as following adaptation to alternating direction motion. This V5/MT activation has been interpreted as the neural correlate of the perceptual motion aftereffect. However, in this issue of *Neuron*, Huk and colleagues (Huk et al., 2001) present provocative new findings that will force a reconsideration of this interpretation. Their new findings suggest that V5/MT activation during the MAE may be entirely accounted for by the subjects' enhanced attention or arousal during perception of illusory motion.

First, the authors replicated earlier findings. Subjects adapted to two moving gratings placed on either side of fixation, and brain activity was measured with functional MRI (fMRI) in a delayed test phase when two static gratings were presented (Figure). Activity in V5/MT was elevated when an MAE was perceived (after adaptation to unidirectional motion), compared to a control condition when no MAE was perceived (after adaptation to alternating direction motion). However, Huk and colleagues then show that V5/MT activation no longer occurred if subjects were instead engaged in a psychophysical task of equivalent difficulty in the test phases of both the MAE and control trials. This suggests that the V5/MT activation previously attributed to the perceptual MAE may instead reflect the additional demands on attention or arousal associated with viewing a moving visual illusion compared to a static stimulus.

These results were obtained by making a slight modification to the test stimulus. One of the test gratings moved very slowly outward, approximately an order of magnitude slower than the psychophysically estimated speed of the MAE. Thus, on both MAE and control trials, one of the two test gratings moved very slightly faster than the other (and either with or without a superimposed MAE). Subjects viewed a short series of these test stimuli, and for both MAE and control trials, were asked to judge which grating moved faster. The task proved difficult for subjects because the added motion was close to the psychophysical threshold, but performance was equivalent in MAE and control conditions. Equating performance in this way, as a proxy for equating attention, led to a dramatic change in V5/MT activation. The previously strong activation during passive viewing was abolished when attention was controlled, with equal activation on MAE and control trials. Importantly, a vivid MAE was perceived on MAE trials even though there was no significant V5/MT activation compared to control trials (without MAE). The physical differences in the stimulus presented in the test phase in the second experiment appear not to account for the differences in V5/MT activation. A control experiment shows that V5/MT responses were not saturated by the presence of the slowly drifting test grating, as responses increased when the contrast of the grating was increased. However, in certain circumstances, dynamic and static test stimuli can produce dissociated MAEs (Culham et al., 2000), suggesting an alternate (if less likely) possibility, that the slowly drifting test grating tapped a different level of motion adaptation.

These findings indicate that a large proportion (perhaps all) of the elevated signal in V5/MT during passive viewing of the MAE may be due to effects of attention. Consistent with this, it is well established that manipulating attention during the adaptation phase can influence V5/MT activity and subsequent perception of the MAE (Chaudhuri, 1991; Rees et al., 1997). However, Huk's new findings add the important observation that these strong effects of attention extend to the test phase. The authors note that their use of the term "attention" in this context is deliberately broad, encompassing both nonspecific effects of arousal and task-dependent attentional changes. Indeed, Huk and colleagues show that the elimination of V5/MT activation in the test phase is independent of the exact nature of the behavioral task, as either speed or contrast discrimination tasks produce equivalent effects on V5/MT activation. Thus,